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ON THE EXISTENCE OF A GROWTH-INHIBITING SUBSTANCE IN THE CHINESE LEMON[1]

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It is the purpose of this preliminary paper to point out the probable existence of a growth-inhibiting substance or substances in the stem of Chinese lemon, a variety of the citron (*Citrus medica*), and to discuss some of its effects upon the development of shoots on the cuttings.

Vöchting[2] believed that each isolated stem is the carrier of a "force" which determines polarity.

The older botanists, under the leadership of Sachs, assumed that there was a flow of shoot-forming substances toward the apex and of root-forming substances toward the base in a regenerating cutting. The difficulty in this assumption, so far as the Chinese lemon is concerned, lies in the fact that none of the subapical buds develop. If the apical buds of the cutting develop as the result of the flow of elaborated materials in their direction, it is difficult to see why these materials which flow past subapical buds should not give them a stimulus to develop, though less in degree.

In a series of recent articles, Loeb has turned attention toward the possibility that there are one or more specific substances in the growing stem which determine the course of events in the growth of new shoots and roots in cuttings. He has shown that in *Bryophyllum calycinum* the apical bud prevents the lower ones from growing out, and he concluded that there is an inhibitory substance sent in the

[1] Paper no. 53, Univ. Calif. Grad. Sch. Trop. Agr. and Citrus Exp. Sta., Riverside, California.

[2] Vöchting, H., Organbildung im Pflanzenreich, Bonn, 1878.

direction of the basal buds. He[3] also believes that the reason why the apical bud grows out first is that it is the first bud which is freed from this substance when the stem is cut from the mother plant.

This view receives strong support from studies on the growth of potato sprouts recently published by Appleman.[4] He finds when all the buds on a tuber are subjected to the same external conditions, that the buds on the apical end grow out first, and that they inhibit the growth of the more basal buds. If these apical sprouts are destroyed, or retarded in their normal growth, sprouts will appear from the more basal buds which would otherwise have remained dormant. The cutting of a furrow around a middle or basal bud will cause it to grow out just as quickly as the apical buds of the tuber. If the tuber is cut into transverse slices the inhibitory influence of the apical buds is removed and there is a general growth of buds over the surface of the entire tuber, depending upon the thickness of the slices.

In the following experiments the cuttings were made from young wood of the Chinese lemon and varied in length from 15 to 40 centimeters. They were suspended in covered glass jars or in glass cases in which a moist atmosphere was continually maintained. The experiments were conducted in a culture room at a temperature which had a daily range of not more than one or two degrees centigrade. Illumination was obtained from two large incandescent electric lights fitted with filters of "Trutint" glass. The jars stood upon a revolving table which was driven at a speed of one revolution every three minutes by a small motor.

Shoots of the Chinese lemon which have attained a sufficient degree of maturity retain their vitality for many months after removal from the tree, provided they are kept in a moist atmosphere. The latency of the buds is not so easily broken as in the case of willow; they therefore constitute a more favorable subject for experimentation.

Regeneration of Shoots from Cuttings

When cuttings made from shoots of the Chinese lemon are suspended in a moist atmosphere, shoots develop from buds at the apex of the cuttings, and roots from the basal end. This occurs whether the cuttings are in the normal or inverted position (figs. 1, 2). The cuttings will grow for several months but no shoots will appear except from two or three buds nearest the apical end of the cutting. If the

[3] Loeb, J., The chemical basis of axial polarity in regeneration, Science, n.s., vol. 46, p. 547, 1917.

[4] Appleman, C. O., Physiological basis for the preparation of potatoes for seed, Bull. 212, Maryland Agr. Exp. Sta., 1918.

buds nearest the apex are killed by burning them with a hot glass rod at the time of installing the experiment then the buds immediately below them will develop, but if the apical buds remain functional and develop the lower buds remain dormant. If the sprout from an apical bud be removed after it has reached a length of one centimeter or more, a new sprout will develop from one of the accessory buds but the lower buds remain dormant. If the tip of a sprout be removed as in the left-hand cutting in figure 1, it will continue to grow from one of its lateral buds but the interruption of its growth does not cause the buds below it to develop.

Similar behavior is shown by shoots on the Chinese lemon tree itself. Figure 3 shows four upright shoots, averaging one meter high, which were taken from a Chinese lemon tree. A few lateral buds near the apex of each shoot had developed into short branches, yet the large number of buds below them had remained dormant.

This dominance of the buds nearest the apex of the shoots is so characteristic and so clearly marked that one can only conclude that it is due to some significant internal influence.

We may now turn our attention to some experiments designed to throw light upon the nature of this influence.

Cuttings about 30 centimeters in length were suspended vertically in a glass jar until one or two shoots were produced at the apex. A notch deep enough to remove bark and phloem was then cut immediately above several subapical buds and the cuttings replaced in the jar. After three weeks nearly every healthy bud above which a notch had been made either produced a shoot or showed signs of activity (fig. 1). The unnotched buds remained dormant. The same result was obtained when the cuttings were hung upside down (fig. 2). None of the buds on the control cuttings developed except those immediately back of the apex. The response is often more manifest if the sprout from the apical bud is allowed to reach a length of one or two centimeters before notching the subapical buds.

The next experiment shows what happens when the development of the apical buds is temporarily inhibited by mechanical means.

Cuttings about 30 centimeters in length, of as uniform size as possible, were selected and the upper half of some of them was enclosed in a plaster of Paris cast. They were then suspended vertically in a glass case and placed on the revolving table. In most cases the casts prevented development of the buds. After a little over three weeks some of the cuttings produced a sprout below the plaster cast.

Figure 5 shows two of these cuttings on which the new sprouts were 8 and 11 centimeters in length respectively. The sprout on each cutting was allowed to grow until it reached a length of several centimeters, then the plaster cast was removed and the cutting again suspended in the moist chamber.

The behavior of the cuttings released from the plaster cast was significant. Soon after removing the casts the apical buds of each cutting developed into sprouts in the normal manner. Figure 6 is from a photograph of the two encased cuttings shown in figure 5, and was taken one month after removing the casts. It shows that the shoots, which had previously developed, began to die when the apical buds began to grow. Subsequently the original shoots died, while those from the apical buds grew on normally. The result of this experiment shows two significant facts: (1) the shoots produced from apical buds after removing the casts were not inhibited in any manner by those already present near the middle of the cuttings; and (2), on the contrary, the original shoots near the middle of the cuttings were inhibited in their growth and finally killed when the apical shoots began to grow. The length of the sprout produced by the subapical bud might alter these relations, for example Mogk[5] reported that subapical sprouts in *Vicia faba* seedlings did not inhibit growth of the apical sprouts unless their relative lengths were as three to one.

The results of these experiments seem to us to indicate that there is some substance produced by the growing shoot which travels in the phloem layers toward the basal part of the stem and that it inhibits the development of buds situated nearer the base of the stem. In making a cutting such as that shown at the left in figure 1, we are isolating a piece of a stem whose buds, according to this assumption would have been previously prevented from developing by a substance emanating from the upper shoots or apical buds. If the substance which was included in this cutting remained evenly distributed throughout its length, none of the buds would have developed, but its tendency seems to be to migrate toward the basal end of the cutting.

The question might arise whether the results may not be due to a tendency for elaborated materials to pass upward more than downward, and to induce the development of apical buds only, through an accumulation of nutrients at the apical end. This question seems to be answered in the negative by two observations: (1) so far as known, the movement of substance in the phloem is downward; (2) the shoots

[5] Mogk, W., Untersuchungen über Korrelation von Knospen und Sprossen, Arch. f. Entwickelsmech. d. Org., vol. 38, pp. 584–681, 1914.

produced from short cuttings having only one or two buds were as large and grew as fast as those produced from the first or second apical bud of a longer cutting.

It cannot be assumed, however, that the movement of this hypothetical substance is directed solely by the pull of gravity. As shown by the growth of cuttings such as illustrated in figure 2, it moves from apex to base even when the cutting is suspended inverted, in a vertical position. It would appear that the substance moves primarily in a basal direction, but that it is influenced to some extent by the pull of gravity.

The formation of new shoots on horizontal branches or on horizontally placed cuttings of Chinese lemon, affords some significant illustrations supporting the assumptions concerning the existence of a growth-inhibiting substance. The horizontal shoot on a tree behaves very differently from vertical shoots, such as those shown in figure 3. Whereas very few lateral buds develop on a vertical shoot, a great many buds develop on a horizontal shoot, provided they have reached a sufficient degree of maturity. Figure 4 shows a long shoot which originally grew vertically on the tree. So long as it was vertical none of the lateral buds developed into shoots. It was bent over and tied in a horizontal position. A few months later this photograph was taken and shows a development of lateral buds along nearly the entire length of the shoot. Shoots of the same age left in the vertical position remained like those shown in figure 3. Inspection of the branch will show, furthermore, that the buds which developed are those situated on the dorsal or upper side. Buds located on the lower side of the horizontal shoot did not develop. This was true of branches which grew naturally in a horizontal position, as well as of those which were bent into that position.

Cuttings suspended horizontally in glass cases produced new sprouts only from buds on the upper side, but not from the lower side (fig. 7). The sprouts were not confined to the apical end of the cutting, though they usually appeared first in that region. The growth of sprouts at the apical end exhibited no such signs of dominance over the other buds on the upper side of the cutting as was shown in the case of cuttings suspended vertically.

This development of the dorsal buds of a horizontal Chinese lemon shoot appears to be in conformity with the idea of a growth-inhibiting substance in the shoot. It appears that the substance accumulates on the lower side of the shoot and prevents its buds from developing,

at the same time freeing the upper side and allowing buds on that side to develop into new shoots.

A very striking result was obtained if a horizontal cutting was revolved through an arc of 180 degrees, so that the position of the upper and lower buds was reversed, after sprouts on the dorsal side had attained a length of 6 to 10 centimeters. A new sprout would soon appear from a bud upon what was previously the ventral side of the cutting. As soon as this new sprout began to grow, the original sprouts began to deteriorate and finally died (fig. 8).

It seems possible that the injury to the original sprouts might have been due both to the growth-inhibiting substance originally present in the cutting, and to additional material produced by the new sprouts. At any rate this substance appeared to accumulate at the lower side of the cutting and to inhibit growth in the shoots which had been brought into that position by the revolution of the cutting. It is important to notice that the two original sprouts on this cutting did not respond normally to the geotropic stimulus previous to their death. This would indicate that there were profound changes in the metabolism and in growth reactions of the sprouts.

It should also be noticed that the root which developed on the cutting shown in figure 8 showed no injury following the revolution of the cutting. It responded to its new position by curving and growing downward as before. The influence of this hypothetical substance upon root development is under investigation and will not be discussed here.

Summary

1. Chinese lemon shoots produce very few lateral branches so long as they grow in a vertical position. Cuttings produce two or three new shoots from buds nearest their morphologically apical end. This dominance of growing buds at the apex of a shoot is characteristic and is also shown by inverted cuttings.

2. The dominant influence of the buds nearest the apex may be prevented from reaching lower buds by notching the phloem layers just above each bud. If the apical bud is prevented from developing by mechanical means, lower buds may develop.

3. Horizontal branches or cuttings of this tree produce lateral shoots only from the dorsal or upper side.

4. The theory is advanced that the shoots developing nearest the apex form a substance which is capable of inhibiting the growth of

other buds on the vertical stem. The hypothetical substance appears to move toward the morphologically basal end of a vertical shoot or piece of shoot. It appears to have a strong deleterious effect upon growth and to perpetuate a condition of dormancy in subapical buds. In horizontally placed shoots this substance appears to settle to the lower side of the shoot.

PLATE 3

Fig. 1. Cuttings of Chinese lemon which had been suspended vertically. Cutting at the left produced two new shoots from the buds nearest the apex. Cutting at the right was notched just above several buds, some of which developed into new shoots.

Fig. 2. Cuttings of Chinese lemon, inverted and suspended vertically. Cutting at the left produced new shoots from buds nearest the apical end of the cutting. Cutting at the right was notched by removing bits of bark and phloem just above the buds. Most of the notched buds developed into new shoots.

Fig. 1

Fig. 2

PLATE 4

Fig. 3. Vertical shoots taken from a Chinese lemon tree. Each shoot has a few small branches shortly below the apex, but no others.

Fig. 4. Branch of a Chinese lemon tree which was bent horizontally from an originally vertical position. Buds on the upper side of the branch developed into shoots.

Fig. 3

Fig. 4

PLATE 5

Fig. 5. Cuttings of Chinese lemon suspended vertically. The apical end of two cuttings was enclosed in a plaster cast. In each case a shoot was produced from the first healthy bud below the cast. Control cutting shown at the left.

Fig. 6. The two cuttings shown at the right of figure 5. Photograph made one month after removal of the casts. The subapical shoots shown in figure 5 died after new shoots developed from the apical buds.

Fig. 5

Fig. 6

PLATE 6

Fig. 7. Cuttings of Chinese lemon suspended horizontally. Buds on the upper side developed into shoots.

Fig. 8. Cutting of Chinese lemon suspended horizontally. After it had produced two new shoots from the upper side the cutting was revolved through an arc of 180°, so that the position of the upper and lower buds was reversed. A new shoot was produced from what was then the upper side and the two original shoots died.

Fig. 7

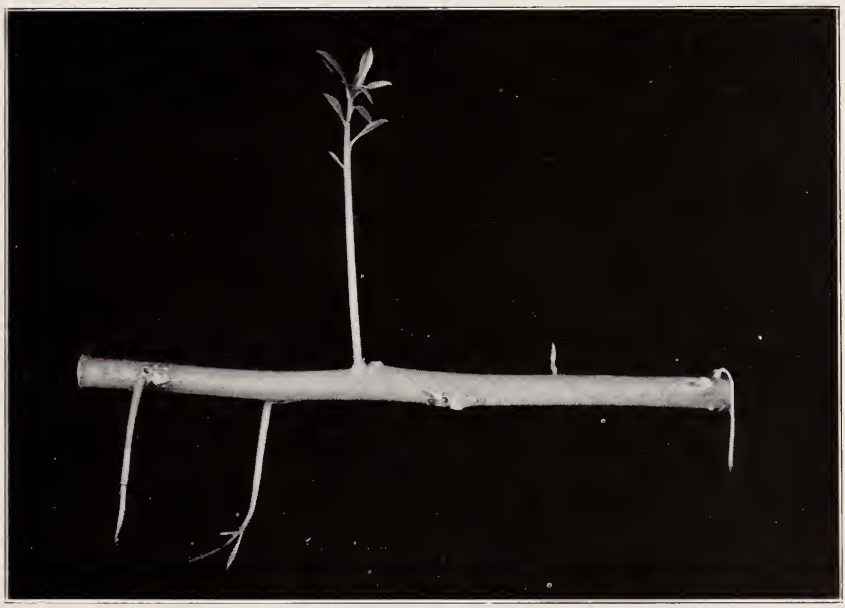

Fig. 8